THE REACTIONS OF DIDINIUM NASUTUM (STEIN) WITH SPECIAL REFERENCE TO THE FEEDING HABITS AND THE FUNCTION OF TRICHOCYSTS.[1]

S. O. MAST.

OUTLINE.

Didinium nasutum has been known for more than a century. It was described by O. F. Müller in 1786 under the name of *Vorticella nasuta*. In 1859 Stein again described this creature and gave it the now generally accepted name, *Didinium nasutum*. Balbiani (1873), however, seems to have been the first to study and record its peculiar feeding habits and his account, although wrong in many respects, had been generally accepted until the paper of Thon (1905) appeared. This paper, however, as the title indicates, deals largely with the structure of the animal and contains only a brief account of the feeding habits. Since this account as well as that of Balbiani seems questionable, I decided to study the reactions of this interesting organism more in detail than had previously been done.

In order to present the results of this study[2] clearly, it will be necessary to give a brief description of the organism.

[1] From the Laboratory of Experimental Zoölogy, Johns Hopkins University.

[2] The experiments forming the basis of this paper were performed in the Zoölogical Laboratory of Johns Hopkins University, in connection with other work, an account of which will be published later. I am much indebted to the university for exceptional facilities put at my command and for financial aid extended to me as Johnston Scholar.

Description.

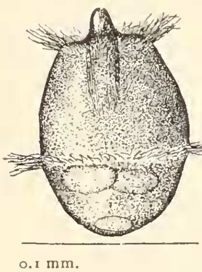
Didinium nasutum is a ciliate protozoön considerably smaller than *Paramecium* (Figs. 1–4). It can scarcely be seen with the naked eye. It is approximately ellipsoidal in form and grayish in color. One end, the anterior, is slightly flattened. From the middle of this end there arises a conical protuberance at the apex of which the mouth is situated. Two distinct bands of cilia encircle the body, one near the middle and the other very near the anterior end. Near the posterior end there is a contractile vacuole and near the central part of the body a relatively large nucleus, which is frequently bent upon itself something like a figure eight. The conical projection appears fibrous. It contains numerous rod-like structures, many of which extend almost to the center of the body. These rod-like structures can be isolated by crushing the animal. They are insoluble in water and appear to be quite rigid. Some of these structures are imbedded in the protoplasm near the surface and appear to serve as support for the conical protuberance, especially during the process of swallowing. The rest are centrally located. Taken together, they form a cylindrical structure referred to by Thon as "mittlerer Strang." *Didinium* seizes and holds its prey by means of this structure. I shall therefore call it the seizing organ.

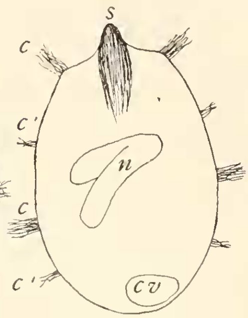
Didinium nasutum is occasionally found in cultures containing *Paramecia;* it usually appears after most of the *Paramecia* have died out, but is seldom very abundant in such cultures. There is, however, little difficulty in obtaining large numbers by judicious feeding on *Paramecia*, which it captures and swallows entire.

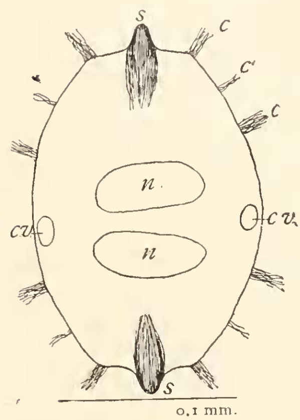
Locomotion.

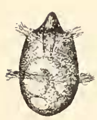
Didinium is a very active creature. One seldom finds it at rest. It swims about rapidly, constantly rotating clockwise on its longitudinal axis and at the same time swerving toward a given side. This causes it to proceed on a spiral course which is usually not over 2 mm. wide. When the organism comes in contact with anything, it ordinarily suddenly reverses the stroke of the cilia, backs a short distance, turns toward one side, and then proceeds on a new course at an angle with the old. That is, it responds with an avoiding reaction similar to that discovered by Jennings in *Paramecium, Oxytricha,* etc. One of the striking

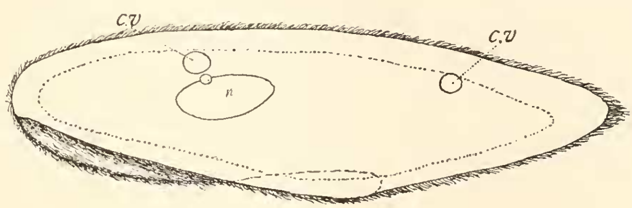
2 3 4











1 5

Fig. 1.[1] *Didinium nasutum*, one of the smallest specimens found. Such specimens result when repeated fission takes place in the absence of food.

Fig. 2. One of the largest not in the process of division.

Fig. 3. One of the largest specimens just beginning the process of fission as indicated by the additional bands of cilia, *c'*; *c,* primary bands of cilia; *n,* nucleus; *c.v.,* contractile vacuole; *s,* seizing organ; 0.1 mm., projected scale.

Fig. 4. One of the largest monsters seen. The narrow bands of cilia, *c'*, indicate the beginning of fission.

Fig. 5. A typical specimen selected from a race of exceptionally large *Paramecia.* Compare with *Paramecium* in Fig. 6.

peculiarities about this creature is the fact that in responding with the avoiding reaction, it always turns toward the same side. One

[1] All the figures accompanying this paper were outlined with the camera from specimens killed suddenly with Worcester's fluid and preserved, after washing with water, by adding ten per cent. glycerine solution and allowing it to concentrate by slow evaporation. No change in size or form could be detected during this process.

would hardly expect this in an organism so nearly radially symmetrical that it is impossible to see any fixed structural differences between the various sides.

That these animals do, however, always turn toward the same side was ascertained in two ways: (1) By withdrawing the water from under the cover-glass until they could no longer swim freely; and (2) by adding Chinese ink to a thin solution of quinced jelly and studying their reactions in this. Under the cover-glass in only a very thin layer of water they could of course turn only in two directions and thus it was very easy to see that they always turned toward the same side under these conditions. When free to swim in all directions, it was much more difficult to follow their movements precisely. In the jelly solution, however, the avoiding reactions are so frequent and the rate of motion is so much reduced that by selecting a specimen to the surface of which particles of ink adhered in such a way as to differentiate the sides, it could be definitely seen that they always turn toward the same side.

By direct observations on the movement of the cilia during the process of turning, and by studying the currents produced, it could be definitely seen that the cilia in the anterior band along the edge toward which the animal turns, strike forward toward the mouth, while the rest in this band and all those in the posterior band strike backward. The fact that some of the cilia in the anterior band strike in one direction at the same time that others in the same band strike in the opposite direction and that this occurs only when the creature is turning, shows an interesting adaptive differentiation in the function of cilia.

In this organism there is no evidence of differential response to localized stimulation. *Didinium* always turns toward the same side, no matter which part of the anterior end comes in contact with an object, the conical projection or various portions of the edge.

Variation in Form.

Not infrequently one finds specimens of *Didinium* which apparently consist of two or three individuals fused together at the posterior ends. These specimens are about two or three times normal size, depending upon the number fused, and all the different parts of normal specimens are duplicated, the bands of cilia, contractile vacuoles, nuclei, seizing organs, etc. When two individuals are fused together, the anterior ends usually point in opposite directions, as represented in Fig. 4, but a few specimens were found in which they pointed in the same direction. Only one specimen composed of three individuals was found. In this the individuals were symmetrically arranged, forming a structure something like a three-pointed star. All these abnormal creatures appear to swim equally well with any one of the anterior ends ahead. They feed just as normal animals do, but I have never found any that reproduced, although at various times isolated specimens were under observation for several days. One specimen, however, was found with two additional bands of cilia indicating that the process of fission had begun (see Fig. 4).

I am unable to account for the origin of these monsters. One might naturally assume it to be a case of imperfect fission, but this is improbable, for in fission an anterior end and a posterior end form at the plane of separation, while in these creatures posterior ends are always in contact.

Variation in Size, Fission and Encystment.

The results of the study of the feeding habits of *Didinium* depend largely upon the relations in size between the *Didinia* under observation that the *Paramecia* upon which they are feeding. Both of these forms vary much more in size than is ordinarily supposed. The smallest *Didinium* obtained (see Fig. 1) measured 0.058 mm. in length and 0.034 mm. in width. The largest normal specimens (Fig. 2) with but two bands of cilia, that is, specimens which were not about to divide, measured 0.130 mm. in length and 0.085 mm. in width. They were therefore more than twice as long and nearly three times as wide as the smaller specimens, which means that the larger are about six times as great as the smaller. Some of the specimens (Fig. 3) about to divide measured 0.166 mm. by 0.101 mm. and one of the largest abnormal specimens (Fig. 4) was 0.207 mm. in length and 0.126 mm. in width, nearly four times as long and four times as wide as the smallest specimens, and about fourteen times as large. *Paramecia* vary nearly if not quite as much in size as *Didinium*. One of the smallest found in the cultures worked with measured 0.117 mm. in length and 0.034 mm. in width and one of the largest was 0.339 mm. long and 0.095 mm. wide, and there were many others of similar size, both small and large, in the cultures. A clear conception of variation in size can be gained by referring to the various figures presented in this article.

The amount of food available is one of the chief factors in regulating the size of *Didinium*. In the absence of food they do not immediately encyst as Thon (p. 293) says, but continue to divide by fission, becoming smaller and smaller after each division. On May 1 at 10:00 A. M. five exceptionally large specimens were taken from a culture and put into a solution without food. At 4:00 P. M. there were six present, one of which was a double monster. May 3 at 9:00 A. M. eleven; May 4, 9:00 A. M., eighteen; May 5, 9:00 A. M., twenty-three; May 6, 9:00 A. M., twenty-six; May 7, 9:00 A. M., twenty. After this the number decreased. I am not positive that they encysted. The monster did not divide at all, consequently twenty-five specimens were produced from four in the total absence of food. One would therefore expect the individuals of the final generation to be much smaller than those of the original. They were in fact about one sixth as large.

These experiments show that size has little if any influence on fission and that absence of food does not primarily cause *Didinium* to encyst. Neither does the presence of food cause these organisms to come out of the cysts. This was clearly shown by adding *Paramecia* to solutions containing encysted *Didinia*. If only a small amount of liquid was transferred with the *Paramecia*, the *Didinia* seldom came out of the cysts; on the other hand, they were induced to come out on several occasions by merely adding water. This seems to indicate that the chemical contents of the water in which the *Didinia* live has much to do with encystment. The relative importance of the various factors involved in this process is, however, a subject for future investigation.

Feeding Habits.

If a number of *Didinia* are studied under a lens, they appear to be darting about in wildest confusion, sharply turning to the right and left, up and down, and frequently running into each other. This is especially true if they have been without food for a few days. In thus rapidly swimming about in every direction, they cover a large space in a short time and come in contact with everything that may happen to be in this space. If a *Didinium* chances to swim against a *Paramecium* in these aimless maneuvers, the two frequently adhere to each other and one soon finds a tangle of fine filaments all about the scene. The whole mass, filaments and all, now moves about as one, but the *Paramecium* disappears in the course of from one to three minutes, having been swallowed by the *Didinium*.

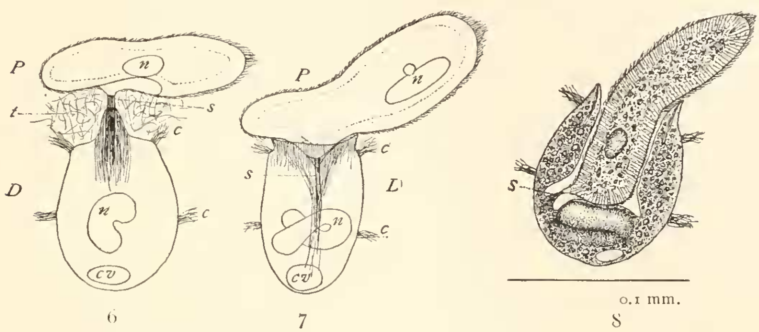


FIG. 6. A large *Didinium* immediately after having seized a small *Paramecium*. *D, Didinium; P, Paramecium; n. nucleus; c,* bands of cilia; *c.v.*, contractile vacuole; *s,* seizing organ, showing small granules seen at the point of contact between the seizing organ and the surface of the *Paramecium; t,* discharged trichocysts; 0.1 mm., projected scale.

FIG. 7. A specimen showing the seizing organ withdrawn and the beginning of the process of swallowing.

FIG. 8. A specimen in which the seizing organ, *S*, could be clearly seen attached to the prey after it had been nearly half swallowed. This shows that the seizing organ travels to the posterior end of the body during the process of swallowing and in so doing no doubt draws in the prey.

Balbiani[1] (1873, p. 363) describes this process as follows: " If while swiftly turning in the water, the *Didinium* happens into the neighborhood of an animalculum, say a *Paramecium,* which it is going to capture, it begins by casting at it a quantity of bacillary corpuscules which constitute its pharyngeal armature. The *Paramecium* immediately stops swimming and shows no other sign

[1] Translation quoted from " Psychic Life of Micro-organisms," by Binet. Published by the Open Court Publishing Company, Chicago, Illinois, 1889, p. 54.

of vitality than feebly to beat the water with its vibratile cilia; on every side of it the darts lie scattered that were used to strike it. Its enemy then approaches and quickly thrusts forth from its mouth an organ shaped like a tongue, relatively long and resembling a transparent cylindrical rod; the free extended extremity of this rod it fastens upon some part of the *Paramecium's* body. The latter is then gradually brought near by the recession of this tongue-shaped organ towards the buccal aperture of the *Didinium,* which opens wide, assuming the shape of a vast funnel in which the prey is swallowed up."

Thon (1905, p. 294) maintains that the *Paramecia* are not killed by trichocysts but by the seizing organ: "Das erste was wir bei der Verfolgung des *Didinium* manövers konstatieren können, ist, dass die Abtötung des *Paramaccium* nicht durch 'Trichocysten' sondern durch den mittleren Strang bewirkt wird. Das lebhaft rotierende *Didinium* tastet mit dem Rande des Mund öffnung an den herumschwimmenden *Paramaecium* umher. Plötzlich schiesst es durch eine energische Kontraktion des Pharynx mit wunderbarer Geschwindigkeit den mittleren Strang hervor, welcher dann aus der Mund öffnung wie ein schlanker, heller Cylinder heraus ragt und manchmal 2/3 Körperlänge erreicht. Dieser Strang wird nun in den Paramaeciumkörper sehr fest eingebohrt und führt den plötzlichen Tod des *Paramaecium* herbei."

Thon is undoubtedly correct in his statement that the *Paramecia* are not killed by trichocysts. As a matter of fact, *Didinium* discharges none. The mass of trichocysts seen after a *Paramecium* is captured by a *Didinium* all come from the *Paramecium,* and not from the *Didinium* as Balbiani and Maupas assumed. This can be readily demonstrated by feeding the *Didinia* organisms which have no trichocysts, *e. g., Colpoda* or *Colpidium.* I have repeatedly seen these protozoa captured and swallowed by *Didinia* but never saw trichocysts discharged during the process. Are the *Paramecia* really poisoned by the seizing organ and suddenly killed as Thon states, and is this organ actually thrust out to seize the prey?

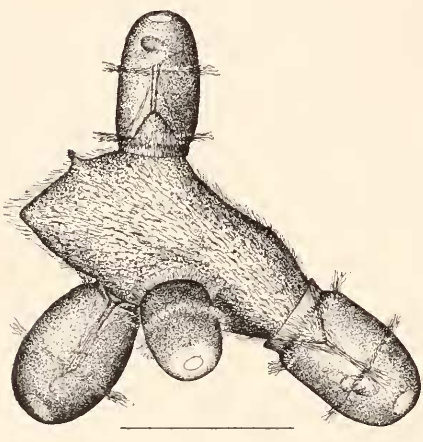
Didinia are powerful swimmers. If one becomes fastened to a *Paramecium* even twice its own size, then the *Paramecium* at once begins to rotate in harmony with the *Didinium,* is carried off and on casual observation it appears to be dead or at least thoroughly paralyzed. If, however, the *Paramecium* is very much larger than the *Didinium* the result is quite different.

During the first part of my work on this subject, I had a race of *Paramecia,* none of which were much more than twice as large as the *Didinia.* After studying the food reactions with these specimens and seeing many *Paramecia* captured and swallowed without any apparent struggle and several, which escaped, die immediately, I was of the opinion that Thon (1905, p. 296) is correct in his statement that the *Paramecia* are poisoned and suddenly killed.

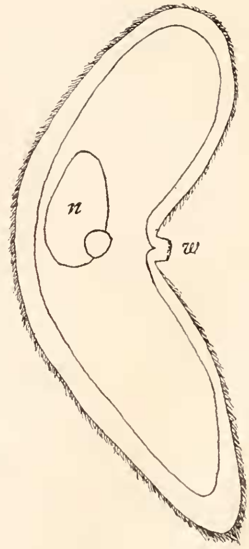
Later, however, there appeared in one of the cultures a race of *Paramecia* which were several times as large as those previously worked on (see Fig. 6, p. 97), and by selecting *Didinia* which had been without food for a few days, it was a simple matter to find specimens not more than one sixth to one tenth as large as these *Paramecia.* These minute *Didinia* attack the huge *Paramecia* as vigorously as they do the smaller ones. In such an attack, however, the scene of action and the result are quite different from that described above. The *Paramecium* attacked does not become quiet, it makes a vigorous struggle and frequently succeeds in throwing off the enemy by breaking the attachment of the seizing organ. I shall discuss the significance of this process later (p. 103). The *Paramecium* thus almost invariably escapes after the first attack and I have frequently seen two and sometimes three *Didinia* successively thrown off before one finally remained attached. And even after one does succeed in this, it merely momentarily checks the movement of the prey. There is no indication of paralysis. The *Paramecium* attacked continues to swim about, carrying the *Didinium* with it. A large specimen studied was thus seen to swim about with a small *Didinium* fastened to its sides for over four minutes, when it was attacked by a second individual, badly wounded and killed. The first individual had swallowed about one fifth of the *Paramecium* before it died, and the second had drawn in a small portion. Both then continued the swallowing process until they had surrounded a large proportion of the body, when the ectosarc gave way and the mouths of the two creatures closed after quite a considerable portion of the substance already swallowed had flowed out.

When the *Didinia* are numerous, several usually attack an animal in rapid succession and it is very soon brought down with a number of these voracious hunters firmly fastened to various portions of the body, as represented in Fig. 9.

Paramecia that escape after having been attacked once or twice very frequently recover. On June 9, at 3:30 P. M., eight such specimens were isolated and put into two watch glasses in a damp



9



10

FIG. 9. A large *Paramecium* attacked by four small *Didinia*. Under such conditions the *Paramecium* is usually torn in pieces and each *Didinium* gets a portion. Sometimes however one *Didinium* gets the entire *Paramecium*, forcing the others off during the process of swallowing.

FIG. 10. A large *Paramecium* which escaped after having been attacked by a *Didinium* showing the wound, *w*, the absence of cilia around the wound, and a marked flexure in the animal, the result of contraction of the ectosarc.

chamber. All the specimens were badly deformed, owing to the violent contractions of the surface surrounding the wound. Three died shortly after they were isolated, leaving two living specimens in one watch glass and three in the other. At 10:00

A. M., June 10, there were three in each watch glass. One of the animals had therefore divided. All appeared nearly normal in form, the bulges and depressions due to the wounds having almost disappeared. At 3:00 P. M., June 11, all in the glass that originally had three were dead, but each of those in the other had divided so that there were now six in the dish that originally had two.

All of these observations show very clearly that *Paramecium* is not immediately paralyzed by *Didinium*, as is generally supposed, and that if there is any poison injected by the seizing organ, it is very mild. There are also other organisms which show no indication of immediate paralysis after an attack by this ciliate. Two of these, *Colpoda* and *Frontonia*, were studied in detail. These protoza appear to have an ectosarc which is not as tough as that of *Paramecium*, so that after the seizing organ is fastened to it and the *Didinium* suddenly reverses its directions of motion and jerks back, as it usually does when it swims against an object, the ectosarc frequently gives way and the enemy swims off with a small mass of protoplasm attached to the seizing organ. Sometimes, however, especially in the case of *Colpoda*, the ectosarc does not break until the *Didinium* has drawn the victim part way in and the mouth begins to close. Thus I have seen specimens of *Colpoda* escape, and recover, after having had various portions, even as much as one third of the body, torn away. And in case these creatures do not escape but are swallowed, the cilia can be seen to beat as long as any part projects.

Frontonia is one of the largest of the protozoa. It is much larger than *Paramecium*, but it is nevertheless, vigorously attacked by *Didinium*. The seizing organ readily sticks to the surface of this creature, but the ectosarc almost always gives way as soon as the *Didinium* jerks back after striking it. This is no doubt due, in part at least, to the relatively great momentum of this animal. It takes a much greater force to move *Frontonia* than it does to move *Colpoda*, so that there is a much greater strain on the ectosarc of the former than there is on that of the latter when a *Didinium* suddenly reverses the direction of motion after the seizing organ is attached. Thus the creature attacked escapes, leaving a portion of the ectosarc with the enemy, which the latter proceeds to swallow.

I am not certain as to how soon *Didinium* is able to make a second attack, but from all appearances it can do so in the course of a few minutes. On May 1, a specimen of *Frontonia leucas* (?) was put into a drop of water on a slide containing numerous *Didinia* which had not been fed during the preceding two days. This specimen was attacked 58 times before it finally died, 40.5 minutes after it was put in. A piece was taken out of the creature at every attack. The wounds thus made immediately closed and the animal swam about as though nothing had happened, but it of course became smaller and smaller, until when it finally burst and died there remained but a minute mass of protoplasm nearly spherical in form, with a diameter nor more than one tenth as great as the original length of the specimen. Four other individuals studied the same day were killed in a much shorter time. They lived after the first attack respectively 6, 7, 10 and 12 minutes. But later, June 9, a specimen lived for nearly two hours after the first attack, and was seized literally hundreds of times by small *Didinia* during this period.

I am well aware that the results of all these observations do not prove conclusively that *Didinium* does not poison its prey, for it may be said that the very fact that a piece remains attached to the seizing organ every time that an animal which has been attacked escapes, saves the animal from being poisoned by preventing any toxic substances from entering the remaining portion of the body. This, however, does not apply to the case where the victim remains alive for some time with *Didinium* fastened to it. As stated above, one specimen lived more than four minutes under such conditions.

Let us now briefly consider the evidence in favor of the view that noxious fluid is used by *Didinium* in capturing its food. This view has its foundation in the apparent sudden paralysis and death of the organism captured, described by Balbiani, Maupas and others. Thon, however, presents other evidence supporting this view. He writes as follows (1905, p. 296): "Dass das Plasma des Stranges auf den Körper der Beute ätzend einwirkt, sehen wir wie an lebenden Tieren so auch besonders an den nach Mallory behandelten Präparaten. Sobald dem *Paramaecium* die Wunde verursacht wird, fangen in der nächsten Umgebung sich zahlreiche Vacuolen zu bilden an, die manchmal eine ansehnliche grösse ereichen und das Plasma quillt hervor." There are then two sources of evidence in favor of the view that noxious fluid is injected by *Didinium* in capturing its prey, the apparent sudden death of the prey and the vacuolation in the neighborhood of the wound made by the seizing organ. We have shown conclusively that paralysis is merely apparent, that organisms are not as suddenly killed as they appear to be, and that they frequently recover if they escape after being wounded. The vacuolation can be conceived to be due to physical effects quite as well as to toxic effects. At any rate it is well known that a physical injury in *Paramecium* does cause vaculation and that these organisms are often killed by slight abrasions. It is almost impossible to obtain specimens which have recovered after having a portion of the body cut off.

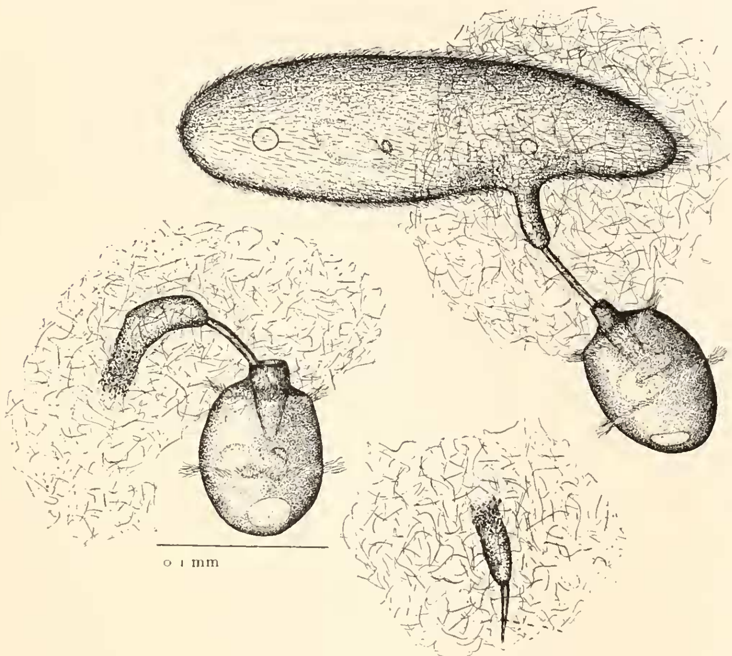
Let us now proceed to the second question stated above. Does *Didinium* thrust out the seizing organ in capturing its prey? This question I shall consider in connection with the function of trichocysts.

Function of the Trichocysts.

If a large number of *Didinia* which have been without food for a few days are thrown into a solution containing numerous *Paramecia,* many of them will begin to feed at once. If they are now suddenly killed, it is not difficult to find specimens attached to *Paramecia* with the seizing organ extended. If the *Paramecia* are relatively small, it will be found to be extended only a short distance, but if they are relatively large, one frequently finds it extended to a distance nearly equal to the length of the body of the *Didinia* as represented in Fig. 11. Balbiani and Thon also represent this organ thus extended. Both of these authors say that it is thrust out. Thon (1905, p. 294) in describing the capture of *Paramecia* says it is forced out by violent contraction with the rapidity of lightning and later (p. 295), in referring to *Didinium* feeding on flagellates, he writes: "Da können wir sehen dass ein *Didinium* in kurzer Frist einiger Minuten eine grosse Zahl von Flagellaten, welche nicht naher bestimmbar ist, einfängt. Blitzschnell wird der Strang hervorgestülpt, mit derselben Geschwindigkeit verschwindet der Flagellat im Innern des Feindes." If this organ was actually shot out with lightning speed, as Thon says, it is difficult to see how one could be certain of just what did happen.

I have studied the maneuvers of *Didinium* in capturing its prey, many times with the greatest care, and was never able to see any indication of such a thrusting out of the seizing organ. What actually happens is this. The *Didinia*, especially when hungry, swim about very rapidly. In such specimens the seizing organ appears to be entirely withdrawn; it cannot be seen to project beyond the surrounding surface in the least. When one of these rapidly moving creatures chances to swim against a *Paramecium*, it strikes with such force that the *Paramecium* can clearly be seen to be pushed out of its course. This is particularly marked if the prey is small. There is no doubt but that the distal end of the oral projection comes in close contact with the surface. This I have actually observed many times. As soon as the *Didinium* strikes an object, *e. g.*, a *Paramecium*, it usually suddenly stops rotating and at the same time reverses its direction of motion. One can never tell whether the seizing organ is fastened or not until this reversion takes place. It very frequently happens that the organ does not become attached during this process and then the *Didinium*, of course, fails to capture its prey. Just why the seizing organ sticks to a *Paramecium* some times and not at others I am unable to explain. All that can be seen in preparations which show the organ fastened is small dots at the point of fusion represented in Fig. 6. It may be that sometimes this organ is entirely withdrawn so that when the hunter strikes a *Paramecium* it does not actually come in contact with the surface.

If the seizing organ becomes fastened, either the fusion with the surface or the pull exerted by the jerking back of the *Didinium* immediately after the fusion produces some sort of injury, for the *Paramecium* at once responds by discharging a great number of trichocysts from the ectosarc for some distance around the point of injury, Fig. 11. As soon as the trichocysts come in contact with the water they form a mass which appears to have a jelly-like consistency; and the increase of this mass due to the extension of the trichocysts forces the two creatures apart, but the attachment between them is not at once broken. The seizing organ draws a portion of the ectosarc out, forming a more or less marked protuberance and it is itself drawn out



12 13 11

FIG. 11. A large *Paramecium* immediately after having been attacked by a small *Didinium*. The discharge of the trichocysts has mechanically forced the *Didinium* back, drawing the seizing organ out and producing a marked protuberance on the surface of the *Paramecium*.

FIG. 12. A *Didinium* entangled in a mass of trichocysts after the *Paramecium* attacked has escaped by tearing off the protoplasmic protuberance.

FIG. 13. A portion of the seizing organ, *S*, left attached to the protoplasmic protuberance after the *Didinium* has escaped. The *Didinium* has become free by twisting off the seizing organ.

at the same time. If the *Didinium* is large and the *Paramecium* small, the discharge of the trichocysts has little effect and one sees but a small protuberance and only a slight extension of the seizing organ, if any; but if the *Didinium* is small and the *Paramecium* large, this organ is frequently drawn out nearly the length of the body, as stated above, and the protuberance of ectosarc becomes so much extended that it breaks and the *Paramecium* swims away, leaving the *Didinium* attached to the bit of ectosarc imbedded in the mass of trichocysts (Fig. 12). It is remarkable what a firm jelly-like consistency this mass has, and how securely the bit of ectosarc pulled out of the *Paramecium* is imbedded in it. The *Didinium* thus fastened to the mass of trichocysts at first appears to be hopelessly entangled but it soon begins to loosen itself by violently jerking backward and darting forward. After it has thus become partly free it begins to rotate on the longitudinal axis and soon twists the seizing organ off at a point in the body some distance from the surface and swims away leaving this important organ in the tangle of trichocysts as represented in Fig. 13. Sometimes the trichocysts give way and the hunter escapes with the bit of protoplasm, which it then swallows. In one such instance, however, which came under my observation, the *Didinium* turned suddenly immediately after it became free, and swam violently against the slide on which it was mounted. The bit of protoplasm adhered to this so firmly that it held the creature fast and it escaped only after twisting the seizing organ off. This illustrates in a striking way one of the properties of bare living protoplasm. I am not positive as to what becomes of these specimens without a seizing organ. They appear normal in every respect and it is altogether likely that the missing organ is regenerated in course of time. There is no evidence that the trichocysts have a toxic effect on the *Didinia*, or that they injure them in any way. They appear to function merely by mechanically separating the prey from its enemy. There are, however, two other factors which assist in this separation: (1) the violent struggle of the *Paramecium* after it is attacked, and (2) the sudden reversion in the direction of motion of the *Didinium*. That these two factors, however, are insufficient in themselves to break the connection or even to account for the extreme extension of the seizing organ and the large protuberance in the ectosarc is evident from the fact that when a *Didinium* attacks a *Paramecium* on a surface where the trichocysts have already been discharged owing to a previous attack, the connection is never broken; the protuberance is insignificant and the seizing organ is only slightly extended if at all. Thus a second hunter frequently succeeds in remaining attached to its prey after the first has been thrown off.

In *Nassula,* a comparatively large ciliate, the trichocysts are of such a nature and so abundant that I have never seen one captured by *Didinium,* although they are vigorously and freely attacked.

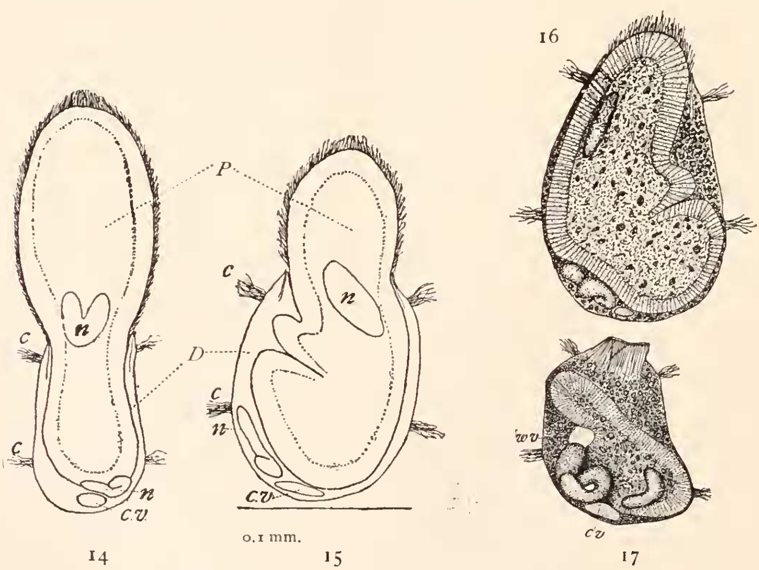
This is, as far as I know, the first instance in which a defensive function of the trichocysts has been actually demonstrated, although it has generally been assumed to be both offensive and defensive. Calkins (1901, p. 50) after referring to the offensive use of these structures in *Didinium,* writes as follows: "This process is strikingly illustrated by the ciliate *Actinobolus radians,* which combines the selection of food with the offensive use of trichocysts. This remarkable organism possesses a coating of cilia and protractile tentacles, which may be elongated to a length equal to three times the body-diameter, or withdrawn completely into the body. The ends of the tentacles are loaded with trichocysts (Entz, 1883). When at rest, the mouth is directed downward, and the tentacles are stretched out in all directions, forming a minute forest of plasmic processes, amongst which smaller ciliates, such as *Urocentrum, Gastrostyla,* etc., or flagellates of all kinds may become entangled without injury to themselves and without disturbing the *Actinobolus* or drawing out the fatal darts. When, however, an *Halteria grandinella,* with its quick and jerky movements, approaches the spot, the carnivore is not so peaceful. The trichocysts are discharged with unerring aim, and the *Halteria* whirls around in a vigorous, but vain, effort to escape, then becomes quiet, with cilia outstretched, perfectly paralyzed." In another section of his book (p. 175), however, referring to the trichocysts in general, Calkins says: "Their function is purely conjectural, although it is generally supposed that they serve as defensive weapons."

Jennings also thinks that we are uncertain as to their function. He writes (1906, p. 90): "They are usually supposed to be weapons of defence. If a *Paramecium* is seized by an animal which is attempting to prey upon it, the trichocysts will of course be discharged from the injured region. But whether they really serve for defence seems questionable. Certainly the infusorian *Didinium*, which is the chief enemy of *Paramecium*, is not hindered in the least from seizing and devouring the animal by the discharge of trichocysts. It is possible that the discharge is really an expression of injury—a purely secondary, even pathological, phenomenon, like the formation of vesicles on the surface of an injured specimen."

It would be of the greatest interest to study the feeding habits of *Actinobolus*, with special reference to the function of trichocysts, in the light of our present knowledge of their function in *Paramecium*.

Mechanics of Swallowing.

It is well known that *Didinia* can capture and swallow organisms which are relatively enormous in size. But it is difficult to obtain the maximum differences in size between the enemy and the victim, for both swim so rapidly that it is impossible to

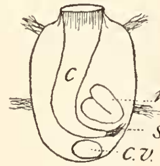


Figs. 14, 15, 16 and 17. Different stages in the process of the swallowing of large *Paramecia* by small *Didinia*. See text. *D*, *Didinium*; *P*, *Paramecium*; *n*, nucleus; *c.v.*, contractile vacuole; *w.v.*, water vacuole.

measure them with any degree of accuracy while alive. and if they are killed before the swallowing process is complete, one can never be certain that it could have been accomplished had they not been killed. The simplest method seems to be, to fix a large number of specimens immediately after they have been feeding, then select the largest and calculate the relative size of the organism swallowed. This was done and some specimens were found in which the substance of the *Paramecium* swallowed occupied approximately ten times as much space as the substance of the *Didinium*. In such specimens the *Didinium* forms a mere film over the *Paramecium*, as represented in Fig. 16. If other animals could engulf objects relatively as large as this hunter ciliate can, a common garter snake could readily swallow a rabbit, a large house cat a sheep, and a lion or a human being a full grown ox.

What are some of the factors involved in the process of swallowing objects of such extraordinary size?

Didinia attack their prey at any point with which they chance to come in contact, and appear to be able to swallow them equally well no matter whether they become attached to the sides or the ends. If a *Didinium* succeeds in remaining attached



18

FIG. 18. A *Didinium* showing a canal, *c*, formed by the invagination due to the movement of the seizing organ, *s*, after the escape of a *Colpoda* which had been partially swallowed. *n*, nucleus; *c.v.*, contractile vacuole.

to its prey after it has made an attack, it at once begins to draw in the seizing organ. This it can do, no matter how far the organ has been extended by the discharge of trichocysts and other factors. The captured prey is thus drawn to the mouth which gradually opens and surrounds it, as the seizing organ travels toward the posterior end of the animal. The whole process is complete in from one to three minutes if the victim is not over twice as large as the *Didinium*.

There are at least four factors involved in this process: (1) The pull exerted by the seizing organ; (2) the suction produced by the active expansion of the body; (3) the extension of the oral opening out over the object; (4) the pressure produced by the contraction of the mouth when the object is nearly swallowed.

1. That the seizing organ travels to the posterior end of *Didinium* and remains attached for some time after the prey is swallowed was shown by Thon (1905, p. 298) by means of sections. In entire specimens mounted in glycerine it can be seen attached to organisms which extend more than half way down the interior of the body (see Fig. 8). The seizing organ returns to its former position after the food is partially digested. If *Didinia* are fed on *Colpoda,* it frequently happens that the ectosarc breaks immediately after the seizing organ is attached to it; then only a very small portion remains attached to the organ, which at once travels to the posterior end of the body drawing the bit of ectosarc with it. This portion of substance is frequently so small that it does not fill the space left by the indrawn organ and in such specimens one can clearly see a marked canal extending from the mouth to the posterior end of the animal, as represented in Fig. 18. The presence of this canal in some of the specimens studied by Balbiani led him to conclude that there is a permanent gut in *Didinium*. It is, however, nothing more than an invagination formed by the movement of the seizing organ and it disappears as soon as this organ returns to its accustomed position. In this canal the food lies. It is therefore in one sense merely in contact with the surface of the body after it is swallowed, much as in *Amœba* where the ectosarc surrounds the food particles and is taken in with it.

2. In some specimens the canal referred to above remains open to the exterior and fills with water as fast as it is formed. In others, however, the oral opening closes as soon as the organ is drawn in and this, of course, prevents the canal from filling. In such specimens, marked depressions are formed in the sides, especially near the anterior end, so that an end view of a *Didinium* in this condition sometimes presents an outline which

is nearly rectangular. This wrinkling of the outer surface indicates that there has been an active expansion; the outer layer of the body has become larger and no longer fits closely over the inner portion. It caves in owing to the decrease in internal pressure. During the swallowing process the extension of the outer layer produces a suction which tends to draw substance in. The fact that one frequently finds spaces filled with liquid (water vacuoles) between the object swallowed and the surrounding wall of the *Didinium,* also indicates that the internal pressure has been decreased. These water vacuoles can be accounted for by assuming that the decreased internal pressure causes the water to leak in around the object as it is passing the oral edge, or by assuming that it has been forced out of the substance already swallowed, owing to its subjection to a decrease in pressure. It is therefore evident that there is an active expansion in the outer portion of *Didinium* and that this makes the internal pressure less than the external and produces suction, which tends to draw the food into the body.

3. That the oral edge moves out to surround the organism captured is evident, but I was not able to ascertain whether this edge creeps out over the body during the swallowing process or whether it is pushed out by the active spreading and extension of the wall back of it.

4. Finally, direct observation shows that the contraction of the oral opening after the prey is nearly swallowed helps to draw over it the *Didinium,* which in this stage of swallowing has the form of a sac. The contraction of the oral opening also forces the prey in at the same time, thus stretching and expanding the thin walls of the *Didinium* more than would be possible if only the inherent spreading forces within these walls were active. The irregularity in the form of a *Didinium* after swallowing a large object supports this conclusion (Fig. 17).

The most marvelous phenomenon in this whole process is the movement of the seizing organ and the active spreading out of the substance of the *Didinium* in the form of a sac-like structure. I know of no other organism with the possible exception of *Hydra* that has the power of such extreme extension.

One can not work long with this unicellular form without being deeply impressed with the complexity of its structure, as well as with its unique habits. The differentiation of the seizing organ with its highly specialized function is remarkable in an organism consisting of a single cell. It shows what we are coming to realize more and more fully as the Protozoa are subjected to more detailed study; namely, that many cells are exceedingly complicated.

Amount and Kind of Food.

The number of *Paramecia* devoured by a *Didinium* in a day depends, of course, upon the size of the *Paramecia*. In all the experiments on this subject the *Paramecia* were between one and two times as large as the *Didinia*. Usually *Didinia* which had been without food for at least a day were selected and put with *Paramecia*. Then after they had swallowed one, they were isolated and later returned from time to time. In this way it was found that they take a *Paramecium* about every three hours and divide two or three times in twenty-four hours. None of the experiments on feeding were, however, continued for more than twelve hours. It is therefore impossible to say that the *Didinia* would feed at the rate mentioned for longer periods.

Didinia which have swallowed a *Paramecium* are, as already stated, frequently very irregular in form, owing to unsymmetrical extension of the body-wall (Fig. 17). As digestion proceeds, such specimens gradually become symmetrical again. I have frequently seen these ciliates capture prey when they were so gorged that they were still very much distorted. This shows that the seizing organ returns to the anterior end of the body long before the creature engulfed is fully digested.

The amount of food that *Didinia* eat depends upon the temperature and various other conditions. Under most favorable conditions they consume relatively enormous quantities. If they should take a *Paramecium*, averaging one and one half times their own size, every three hours, as the experiments described above indicate, in twenty-four hours they would consume a mass of substance having a volume twelve times as great as their own. I know of no other creatures that even approach these in their feeding capacity.

At different times during the progress of this work, *Didinia* were tested as to their ability to capture various organisms. These organisms may be divided into two groups: (1) those which were captured or attacked and (2) those which were not.

Group 1.—Paramecium caudatum, P. aureleus, Colpoda, various sp., *Colpidium,* various sp., *Vorticella,* sp. (?), *Frontonia,* sp. (?), *Nassula,* sp. (?).

Group 2.—Stentor cœruleus, Euglena, various sp., *Spirostomum,* various sp., rotifers, various sp., *Loxophyllum* sp. (?), *Paramecium bursaria, Oxytricha,* various sp., *Stylonychia,* various sp., numerous small flagellates, some identified and some not, *Paramecia* which had been killed by rapid heating.

Of those in the first group all but *Nassula* were at different times seen captured and swallowed by *Didinia. Nassula* was vigorously attacked, but every time the seizing organ became attached the connection was immediately broken by the discharge of trichocysts. I did not, however, work very much with these organisms. It may be that *Didinia* can master them under conditions different from those to which they were subjected during my observations. It will thus be seen that *Didinia* feed on various forms. *Paramecia,* however, seem to be essential to their well-being. I have never been able to get rapid multiplication in their absence.

Choice of Food.

If organisms from both groups mentioned above are present in a culture at the same time, only those belonging to the first group will be taken. Under such conditions it appears as though the *Didinium* had the power of selecting its food, and because of this apparent power of selection *Didinium* is frequently referred to in discussions on the choice of food in protozoa.

In the account of the process of feeding as given by Balbiani (see above, p. 97) it must be assumed that the *Didinia* can in some way distinguish different protozoa at a distance, for he says that these hunters discharge their darts even before they come in contact with the victim. Thon's description (1905, p. 201) also implies the ability to distinguish different infusoria, although not at a distance. "Sehr wichtig sind die Beobachtungen des Zustandes, wenn die Didinien aus Mangel an Paramaecien, gezwungen sind die Flagelleten zu jagen." From this quotation it must be concluded that the flagellates are taken only in the absence of *Paramecium*, never if both forms are present in the same culture. *Didinia* must, therefore, have some method of distinguishing between them.

The assumption of choice based upon Balbiani's description of course falls to the ground, since it has been shown (p. 98) that his observations were erroneous, and Thon unfortunately did not ascertain the species to which the flagellates mentioned belong. It is therefore impossible to repeat his experiments. In all the forms on which I worked it was, however, found that there is no apparent selection among the different forms in the first group. All of them, excepting *Nassula,* were at different times seen captured in the presence of *Paramecia*. In order to emphasize this point, let me quote from my notes written immediately after the observations were made. "On March 7 *Didinia* were put into a drop of solution on a slide containing numerous *Colpoda* and some *Paramecia*. Scarcely any of these creatures were attacked for an hour after the *Didinia* were added, although there were frequent collisions. Finally one *Colpoda* was captured and immediately after this, in the same vicinity, two more and also a *Paramecium*. It is evident then that *Colpoda* and various other forms are taken in the presence of *Paramecia*. This fact, however, does not exclude the possibility of choice even among these. It may be that while various organisms are captured in the presence of *Paramecia,* the number of these taken is relatively small. Whether this is actually true or not can be ascertained only by statistically comparing the number of successful contacts in the various organisms. It is clear that *Didinia* come in contact with their prey by chance. The question is, does the seizing organ adhere to one relatively more often than to another? However this may be, it is certain that *Didinia* come in contact with all sorts of objects and devour all such to which the seizing organ will adhere. The question then remains, why does this organ adhere to the surface of certain forms and not to that of others?

The following quotation presents Jennings' view (1906, p. 185): "On coming in contact with a solid object it (*Didinium*) stops, pushes forward against the object the conical projection which bears the mouth, and revolves rapidly on its long axis. The mouth is armed with a number of strong ribs ending in points, which apparently project a little from the cone bearing the mouth. When pushed forward against a soft organism, these points apparently pierce and hold it. The revolution on the long axis has the appearance of a process of boring into the body. The mouth now opens widely and swallows the prey. . . . The point which interests us at present is that *Didinium* reacts in the way described not merely to objects which may serve as food, but also to all sorts of solid bodies. In other words, the process is one of the trial of all sorts of conditions. On coming in contact with a solid, *Didinium* 'tries' to pierce and swallow it. If this succeeds, well and good; if it does not, something else is 'tried.' In a culture containing many specimens of *Didinium*, the author has seen dozens of individuals reacting in this way to the bottom and the sides of the glass vessel, apparently making persevering efforts to pierce the glass. Others 'try' water plants, or masses of small algæ, about which many specimens gather at times. Of course they get no food in this way. On coming in contact with each other, the animals react in the same way, often becoming attached to each other, and sometimes forming chains of four or five. But they never succeed in swallowing one another. They often try rotifers in the same way but the outer integument of these organisms is so tough that *Didinium* does not succeed in piercing it, and the rotifer escapes. *Stentor* and *Spirostomum* are often fastened upon, but usually escape, owing to their large size, great activity and rather tough outer covering. The reason why *Paramecium* is usually employed as food rather than other organisms is clearly due to the fact that when the *Didinia* try these, they usually succeed in piercing and swallowing them while with most other objects they fail."

It will thus be seen that Jennings emphasizes three factors in discussing the process of fastening onto the prey: (1) the rotation of *Didinium* on its long axis; (2) the size and activity of the organism attacked; (3) the toughness of the outer covering of these organisms.

1. There is no doubt about the fact that these creatures are frequently seen rapidly rotating on the long axis with the anterior end against objects of all sorts, but I feel very certain that this reaction has nothing to do with the process of capturing food, for I have repeatedly seen specimens which had just swallowed a *Paramecium* performing this very act. As a matter of fact, after having swallowed its prey *Didinium* ordinarily swims down and when it comes in contact with the bottom it remains there rotating on its axis, so that shortly after feeding one can frequently see many of them with the anterior end against the bottom, spinning much like miniature tops. Specimens which had just been fed were also repeatedly seen swimming about in contact with each other, sometimes as many as five or six in series, like a string of beads, each one apparently trying to seize the one in front of it.

One never, or rarely at least, sees this reaction in specimens which have been without food. Such specimens immediately respond with the motor reaction when they come in contact with an object. They appear to be darting about in every direction in wildest confusion, never coming to rest until they are actually attached to their prey. This attachment, as previously stated (p. 104), takes place at the very instant the *Didinium* strikes the surface to which it becomes fastened and never after having been in contact for some time apparently boring into the surface. The boring reaction may then be considered to be due to inactivity. *Didinium* responds in this way when the threshold of stimulation is increased to such an extent that contact no longer induces the avoiding reaction.

2. The size and the activity of the organisms are important factors in the general process of feeding, but they have nothing to do with the question as to the cause of the attachment of the seizing organ, for it readily adheres to *Frontonia* which are much larger than *Paramecia* and it does not adhere to *Euglenæ* and specimens of *Spirostomum,* which are much smaller.

3. The character of the outer surface seems to be the controlling factor in determining the possibility of the attachment of the seizing organ and this, together with the trichocysts and the size and activity of the organism, determines whether or not it

can escape the attack of *Didinium*. I am inclined to believe that the chemical composition as well as the physical constitution ha. to do with this process, that it is not merely the toughness of the integument but also the chemical composition of this covering that prevents the seizing organ from sticking. One thing is certain: it does not stick to the surface of any of the forms mentioned under group 1 (p. 113), and surely the outer covering of some of these, *e. g., Oxytrica, Euglena,* etc., is no tougher than that of *Paramecium* or *Vorticella*.

Is the conclusion of Jennings that *Didinia* try all objects and feed on any they can master, valid? I think there can be no doubt but that it is. This conclusion, however, has nothing to do with the question of conscious choice. Neither does the fact that the apparent choice has been explained mechanically show that *Didinium* is devoid of consciousness. For all that is known to the contrary, *Didinium* may be aware of the difference between different organisms. It may have certain sensations when it comes in contact with one surface and others when it comes in contact with another. I am not assuming that this organism is conscious, nor am I assuming that it is not. The question as to consciousness in this creature is open and should be left open until we have much more evidence bearing on it than we now have.

Summary.

1. When *Didinium* comes in contact with an object, it usually responds with the avoiding reaction. In this response it always turns toward the same side, in spite of the fact that it is practically radially symmetrical.

2. It feeds entirely upon living organisms, principally *Paramecium,* but it has been known to devour also *Colpoda, Colpidium, Frontonia* and *Vorticella*. It captures these organisms by accidentally coming in contact with them in swimming about at random. The prey is held by means of the seizing organ, which in some way adheres to the surface when the contact is made.

3. There are no trichocysts discharged by the *Didinia*, they come from the victims; neither is the seizing organ thrust out at

the prey, nor is the prey paralyzed by poison injected through this organ.

4. The trichocysts function as organs of defence. They are discharged in great numbers when the seizing organ becomes fastened to the ectosarc. This forces the *Didinium* back mechanically and frequently breaks the connection, thus setting the victim free.

5. Well fed *Didinia* continue to divide for some time without food, becoming smaller and smaller until they are not more than one tenth the size of the original one.

6. Encysted *Didinia* are frequently found. The chemical composition of the solution in which they live seems to cause them to encyst, rather than lack of food, as maintained by Thon.

7. The apparent choice of food is due to the fact that the seizing organ will adhere to the surface of some organisms and not to that of others. The *Didinia* come in contact with all sorts of objects in their random swimming and attempt to swallow all of those to which the seizing organ will adhere.

LITERATURE CITED.

Balbiani, E. G.

1873 Observations sur le Didinium nasutum. Arch. d. Zool. Exp., Vol. 2, pp. 363–394.

Calkins, Gary N.

1901 The protozoa. New York. 347 pp.

Jennings, H. S.

1906 The Behavior of the Lower Organisms. New York. 366 pp.

Thon, Karel.

1905 Ueber den feineren Bau von Didinium nasutum, O. F. M. Protistenkunde, Vols. 5–6, pp. 280–315.